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חוק הפטנטים, תשכ"ז-1967
PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

לשימוש הלשכה
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אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
(Name and address of applicant, and in case of body corporate-place of incorporation)

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(באנגלית)
(English)

WATER-SOLUBLE BACTERIOCHLOROPHYLL DERIVATIVES AND THEIR PHARMACEUTICAL USES

מבקש בזאת כי ינתן לי עליה פטנט
hereby apply for a patent to be granted to me in respect thereof.

*בקשת חלוקה - Application of Division	*בקשת פטנט מוסף - Application for Patent Addition	*דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	*לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
מס'/No.	מס'/No.			
מיום/dated	מיום/dated			

* יפוי כח: כללי / מיוחד - רצוף בזה / עוד יוגש
P.O.A.: general / individual-attached / to be filed later -

הוגש בעניין/filed in case

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חתימת המבקש
Signature of Applicant

For the Applicants,
Paulina Ben-Ami
Patent Attorney

היום 17 בחודש נובמבר שנת 2002
This 17 of November of the year 2002

לשימוש הלשכה
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REFERENCE: STEBA-006 IL סימוכין:

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STEBA-006 IL

תולדות בקטריוכלורופיל המסיסות במים ושימושים פרמאצבטיים שלהן

WATER-SOLUBLE BACTERIOCHLOROPHYLL DERIVATIVES AND THEIR PHARMACEUTICAL USES

YEDA RESEARCH AND DEVELOPMENT CO.LTD. **ידע חברה למחקר ופתוח בע"מ**

Inventors: Avigdor Scherz, Alexander Brandis, Ohad Mazor, Yoram Salomon, Hugo Scheer

ממציאים: אביגדור שרץ, אלכסנדר ברנדיס, אוהד מזור, יורם סלומון, הוגו שר

WATER-SOLUBLE BACTERIOCHLOROPHYLL DERIVATIVES AND THEIR PHARMACEUTICAL USES

FIELD OF THE INVENTION

The present invention relates to novel water-soluble derivatives of bacteriochlorophyll, to their preparation and their use in methods of *in vivo* photodynamic therapy and diagnosis of tumors and different vascular diseases such as age-related macular degeneration, as well as in killing of viruses and microorganisms.

DEFINITIONS AND ABBREVIATIONS

AMD: age-related macular degeneration; **Bchl:** bacteriochlorophyll a; **Bchlorin:**bacteriochlorin (7,8,17,18-tetrahydroporphyrin); **Bphe:** bacteriopheophytin a (Bchl in which the central Mg atom is replaced by two H atoms); **Bpheid:** bacteriopheophorbide a (the C-17^2-free carboxylic acid derived from BPhe); **Pd-Bpheid:** Pd-bacteriopheophorbide a; **PDT:** photodynamic therapy; **Rhodobacteriochlorin:** Bchlorin having a -CH_2CH_2COOH group at position 17, a -COOH at position 13, a methyl group at positions 2, 7, 12, and 18, and an ethyl group at positions 3 and 8.

IUPAC numbering of the bacteriochlorophyll derivatives is used throughout the specification. Using this nomenclature, the natural bacteriochlorophylls carry two carboxylic acid esters at positions 13^2 and 17^2, however they are esterified at positions 13^3 and 17^3.

BACKGROUND OF THE INVENTION

Photodynamic therapy (PDT) is a non-surgical treatment of tumors in which non-toxic drugs and non-hazardous photosensitizing irradiation are combined to generate cytotoxic reactive oxygen species *in situ*. This technique is more selective than the commonly used tumor chemotherapy and radiotherapy. To date, porphyrins have been employed as the primary photosensitizing agents in clinics. However, current sensitizers suffer from several deficiencies that limit their application, including mainly: (1) relatively weak absorption in the visible spectral range which limits the treatment to shallow tumors; (2) accumulation and long retention of the

sensitizer in the patient skin, leading to prolonged (days to months) skin phototoxicity; and (3) small or even no differentiation between the PDT effect on illuminated tumor and non-tumor tissues. The drawbacks of current drugs inspired an extensive search for long wavelength absorbing second-generation sensitizers that exhibit better differentiation between their retention in tumor cells and skin or other normal tissues.

The spectra, photophysics, and photochemistry of native bacteriochlorophylls (Bchls) have made them optimal light-harvesting molecules with clear advantages over other sensitizers presently used in PDT. In particular, these molecules have a very high extinction coefficient at long wavelengths (λ_{max}=760-780 nm, ε=(4-10)$\times 10^4$ $M^{-1}cm^{-1}$), where light penetrates deeply into tissues. They also generate reactive oxygen species (ROS) at a high quantum yield (depending on the central metal).

The biological uptake and PDT efficacy of metal-free derivatives of Bchl have been studied with the objective to manipulate the affinity of the sensitizers to the tumor cellular compartment. Cardinal to this approach is the use of highly lipophilic drugs that may increase the accumulation of the drug in the tumor cells, but also renders its delivery difficult. In addition, the reported biodistribution shows significant phototoxic drug levels in non-tumor tissues over prolonged periods (at least days) after administering the drug.

In applicant's previous Israel Patent No. 102645 and corresponding US Patents US 5,726,169, US 5,726,169, US 5,955,585 and US 6,147,195, a different approach was taken by the inventors. Highly efficient anti-vascular sensitizers that do not extravasate from the circulation after administration and have short lifetime in the blood were studied. It was expected that the inherent difference between vessels of normal and abnormal tissues such as tumors or other tissues that rely on neovessels, would enable relatively selective destruction of the abnormal tissue. Hence, it was aimed to synthesize Bchl derivatives that are more polar and hence have better chance to stay in the vascular compartment, where they convey the primary photodynamic effect. To this end, the geranylgeranyl residue at the C-17 position of Bchl a (Scheme 1, Compound 1) has been replaced by various residues such as amino acids, peptides, or proteins, which enhance the sensitizer hydrophilicity. One particular derivative, Bchl-Ser (Scheme 1, Compound 1 wherein R is seryl), was found to be water-soluble and highly phototoxic in cell cultures. Following intraperitoneal (i.p.) injection, the Bchl-Ser cleared from the mouse blood and tissues bi-exponentially in a relatively

short time ($t_{1/2}$ ~2 and 16 h, respectively). Clearance from the circulation was even faster following intravenous injection. Under the selected treatment protocol (light application within minutes after drug injection), phototoxicity was predominantly conferred to the tumor vasculature (Rosenbach-Belkin et al., 1996; Zilberstein et al., 2001 and 1997). However, unfortunately, like native Bchl, the Bchl-Ser derivative undergoes rapid photo-oxidation, forming the corresponding 2-desvinyl-2-acetyl-chlorophyllide ester and other products.

To increase the stability of the Bchl derivatives, the central Mg atom was replaced by Pd in the later applicant's PCT Publication WO 00/33833. This heavy atom was previously shown to markedly increase the oxidation potential of the Bchl macrocycle and, at the same time, to greatly enhance the intersystem-crossing (ISC) rate of the molecule to its triplet state. The metal replacement was performed by direct incorporation of Pd^{2+} ion into a Bpheid molecule, as described in WO 00/33833. Based on the pigment biodistribution and pharmacokinetics, it was assumed that the derivative Pd-Bpheid remained in the circulation for a very short time with practically no extravasation to other tissues, and is therefore a good candidate for vascular-targeting PDT that avoids skin phototoxicity. The treatment effect on the blood vessels was demonstrated by intravital microscopy of treated blood vessels and staining with Evans-Blue. Using a treatment protocol with a minimal drug-to-light interval, Pd-Bpheid was shown to be effective in the eradication of different tumors in mice, rats and other animal models and is presently entering Phase I/II clinical trials in patients with prostate cancer that failed radiation therapy.

Because of its low solubility in aqueous solutions, the clinical use of Pd-Bpheid requires the use of solubilizing agents such as Cremophor that may cause side effects at high doses. It would be highly desirable to render the Pd-Bpheid water-soluble while retaining its physico-chemical properties. Alternatively, it would be desirable to prepare a Pd-Bpheid derivative that is cytophototoxic and, at the same time, is more water-soluble than Pd-Bpheid itself. Such water solubility is expected to further enhance the drug retention in the circulation and, thereby, the aforementioned selectivity. In addition, having no need to use carriers such as detergents or lyposomes, may prevent side effects.

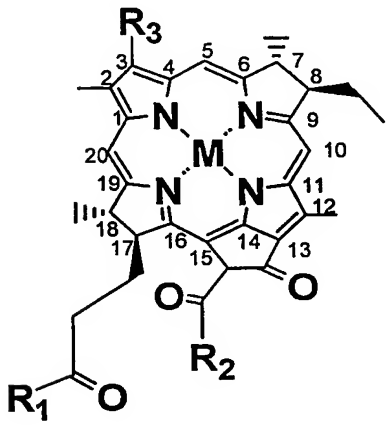
SUMMARY OF THE INVENTION

It has now been found in accordance with the present invention that bacteriochlorophyll derivatives containing at least two negatively charged groups and/or acidic groups that are converted to negatively charged groups at the physiological pH, are water-soluble and thus can be more suitable for pharmaceutical use than the known partially water-soluble Bchl-Ser and Pd-Bpheid.

The present invention thus relates to a bacteriochlorophyll derivative containing at least two, preferably two or three, negatively charged groups and/or acidic groups that are converted to negatively charged groups at the physiological pH, excluding the compounds devoid a central metal atom and having a $-CH_2CH_2COOH$ group at position 17, a $-CH_2COOH$ or –COOH group at position 15, a -COOH group at position 13, a methyl group at each of the positions 2, 7, 12, and 18, and an ethyl group at each of the positions 3 and 8.

The negatively charged groups include, but are not limited to, COO^-, COS^-, SO_3^-, and PO_3^{2-}, and the acidic groups include COOH, COSH, SO_3H and PO_3H_2, that are converted to COO^-, COS^-, SO_3^-, and PO_3^{2-}, respectively, at the physiological pH.

In one embodiment, the bacteriochlorophyll derivative has the formula I or II:



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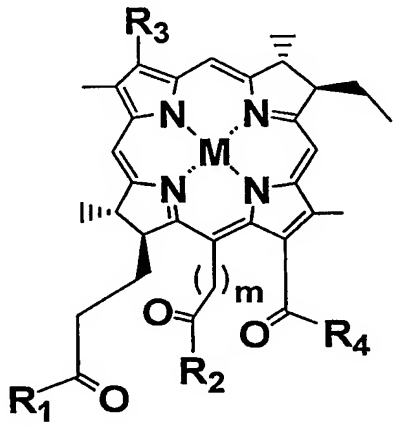


(II)

wherein

M represents 2H or a metal atom selected from divalent Pd, Pt, Co, Sn, Ni, Cu, Zn and Mn, and trivalent Fe, Mn and Cr;

R_1, R_2, and R_4 each independently is Y- R_5;

Y is O, S or NR_5R_6;

R_3 is selected from $-CH=CH_2$, $-C(=O)-CH_3$, $-C(=O)-H$, $-CH=NR_7$, $-C(CH_3)=NR_7$, $-CH_2-OR_7$, $-CH_2-SR_7$, $-CH_2-NR_7R'_7$, $-CH(CH_3)-OR_7$, $-CH(CH_3)-SR_7$, $-CH(CH_3)-NR_7R'_7$, $-CH(CH_3)Hal$, $-CH_2-Hal$, $-CH_2-R_7$, $-CH=CR_7R'_7$, $-C(CH_3)=CR_7R'_7$, $-CH=CR_7Hal$, $-C(CH_3)=CR_7Hal$, and $-C\equiv CR_7$;

R_5 , R_6 , R_7 and R'_7 each is independently H; a straight or branched, saturated or unsaturated, substituted or unsubstituted hydrocarbon chain having from 1 to 25 carbon atoms and optionally interrupted by heteroatoms and/or carbocyclic or heterocyclic moieties; a residue of an amino acid, a peptide or of a protein; and, when Y is O or S, R_5 may further be H or R_8^+;

m is 0 or 1;

R_8^+ is H^+ or a cation;

provided that: (i) at least two of R_5, R_6, R_7 and R'_7 are such a hydrocarbon chain substituted by a negatively charged group or by an acidic group that is converted to a negatively charged group at the physiological pH and/or a residue of an amino acid, a peptide or of a protein; or (ii) at least two of R_1, R_2, and R_4 are OH, SH, $O^- R_8^+$ or $S^- R_8^+$; or (iii) at least one of R_1, R_2, and R_4 is OH, SH, $O^- R_8^+$ or $S^- R_8^+$ and at least one of R_5, R_6, R_7 and R'_7 is such a hydrocarbon chain substituted by a negatively charged group or by an acidic group that is converted to a negatively charged group at the physiological pH and/or a residue of an amino acid, a peptide or of a protein, but excluding the compounds of formula II wherein M is 2H, R_3 is $-C(=O)CH_3$, R_1, R_2 and R_4 are OH, and m is 0 or 1.

The invention further relates to pharmaceutical compositions comprising a bacteriochlorophyll derivative as defined above for photodynamic therapy (PDT), particularly for vascular-targeting PDT, for example for PDT of tumors or of age-related macular degeneration (AMD), or for killing cells or infectious agents comprising bacteria and viruses *in vivo* or *in vitro*, as well as for diagnostic purposes.

The invention provides a method for photodynamic therapy using a photosensitizer, wherein the improvement consists in that said photosensitizer is a bacteriochlorophyll derivative of the invention. According to this aspect, the invention relates to a method for treatment by PDT which comprises administering to an individual in need an effective amount of a bacteriochlorophyll derivative of the invention, followed by local irradiation.

The invention further provides a method for for diagnosis of tumors using a photosensitizer, wherein the improvement consists in that said photosensitizer is a bacteriochlorophyll derivative of the invention. According to this aspect, the invention relates to a method for diagnosis of tumors which comprises administering to an individual suspected of having a tumor an effective amount of a bacteriochlorophyll derivative of the invention, followed by local irradiation.

The invention still further provides a method for killing cells or infectious agents comprising bacteria and viruses, using a photosensitizer, the improvement wherein said photosensitizer is a bacteriochlorophyll derivative of the invention. According to this aspect, the invention relates to a method for sterilization of biological products, e.g. blood, which comprises adding to said biological product, e.g. blood, an effective amount of a bacteriochlorophyll derivative of the invention, followed by irradiation.

BRIEF DESCRIPTION OF THE FIGURES

The different compounds tested are represented in the following description of the drawings by a bold and underlined numeral. Their full identification is found in the List of Compounds at the beginning of the Chemical Section hereinafter.

Figs. 1A-1B are graphs showing the phototoxicity of the sulfonated compound 8 on H5V mouse endothelial cells (Fig. 1A) and M2R mouse melanoma cells (Fig. 1B). Cells were incubated with increasing concentrations of 8 for 4 hours, washed and illuminated (open shapes) or kept in the dark (dark control, closed shapes). Points are average of triplicates ± STD.

Figs. 2A-2B are graphs showing the phototoxicity of the sulfonated compound 4 on H5V mouse endothelial cells (Fig. 2A) and M2R mouse melanoma cells (Fig. 2B). Cells were incubated with increasing concentrations of 4 for 4 hours, washed and illuminated (open shapes) or kept in the dark (dark control, closed shapes). Points are average of triplicates ± STD.

Fig. 3 is a graph showing the phototoxicity of the sulfonated compound 5 on M2R mouse melanoma cells. Cells were incubated with increasing concentrations of 5 for 4 hours, washed and illuminated (circles) or kept in the dark (dark control, diamonds). Points are average of triplicates.

Fig. 4 is a graph showing the phototoxicity of the sulfonated compound 11 on M2R mouse melanoma cells. Cells were incubated with increasing concentrations of

11 for 4 hours, washed and illuminated (circles) or kept in the dark (dark control, diamonds). Points are average of triplicates.

Fig. 5 is a graph showing pharmacokinetics of compound 4 in CD1 nude mice blood. Following pigment 4 injection (6 mg/kg), blood samples were collected from the same mouse at the indicated times and Pd was determined. Each time point represents average of three mice ± STD.

Fig. 6 shows biodistribution of 4 in CD1 nude mice. Mice were sacrificed at different times following 4 injection (6 mg/kg), and Pd content was determined for the indicated organs. Each time point represents average of three mice ± STD.

Figs. 7 shows PDT of melanoma xenografts with 4. Mice bearing M2R melanoma xenografts were intravenously injected with 4 (6 mg kg^{-1}) and illuminated for 5 min with light intensity of 30J/cm^2 (n=14, filled squares), 39J/cm^2 (n=8, filled diamonds) or 45J/cm^2 (n=10, filled triangles). Mice that were injected with 9 mg kg^{-1} of 4 were illuminated for 5 min with 30J/cm^2 (n=10, filled circles). Control groups: untreated (n=4, open squares), dark control received 6 mg kg^{-1} (n=4, open circles) or 9 mg kg^{-1} (n=5, open triangles) of 4, and light control (n=6, open diamonds, 45J/cm^2).

Figs. 8a-8h are photographs showing the selective effect of PDT in mice bearing rat C6 glioma xenografts and treated with 4. (a-d) PDT treated animal; (e-h) untreated animal. (a) before treatment; (b) 3 hours after PDT and Evans-Blue (EB) injection; (c) skin flap of the treated area, 24 hours after PDT; (d) axial slice of the treated tumor 24 hours after PDT; (e) before EB injection; (f) 3 hours after EB injection; (g) skin flap 24 hours after EB injection; (h) axial slice of the untreated tumor, 24 hours after EB injection. T-tumor; S-skin; M-muscle; E-edema.

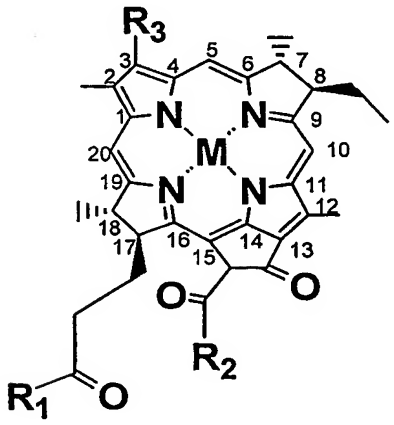
DETAILED DESCRIPTION OF THE INVENTION

The present invention provides, in a broad aspect, bacteriochlorophyll derivatives containing at least two, preferably 2 or 3, negatively charged groups and/or acidic groups that are converted to negatively charged groups at the physiological pH, but excluding the compounds devoid a central metal atom and having a -CH_2CH_2COOH group at position 17, a -CH_2COOH or –COOH group at position 15, a -COOH group at position 13, a methyl group at each of the positions 2, 7, 12, and 18, and an ethyl group at each of the positions 3 and 8.

The bacteriochlorophyll derivatives may be derived from a natural or synthetic derivative of bacteriochlorophyll, including compounds in which the central Mg atom has been deleted or replaced by other metal atoms such as divalent Pd, Pt, Co, Sn, Ni, Cu, Zn and Mn, and trivalent Fe, Mn and Cr. In one preferred embodiment, the metal atom is Pd.

Any suitable negatively charged group and/or acidic groups that are converted to negatively charged groups at the physiological pH may be used in the invention such as, but not limited to carboxylate (COO^-), thiocarboxylate (COS^-), sulfonate (SO_3^-), and phosphonate (PO_3^{2-}) and/or the acidic groups carboxylic (COOH), thiocarboxylic (COSH), sulfonic (SO_3H) and phosphonic (PO_3H_2) acid groups, from which said charged groups originate. These groups may be present as a substitution in a group in any suitable position in the periphery of the bacteriochlorophyll molecule, preferably in two of the positions 3, 13, 15 and 17.

In one embodiment, the invention provides a bacteriochlorophyll derivative of the formula I or II:



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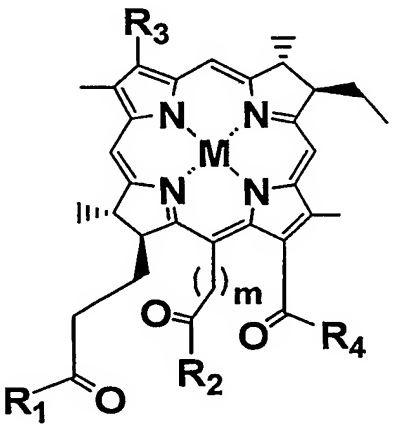

(II)

wherein

M represents 2H or a metal atom selected from divalent Pd, Pt, Co, Sn, Ni, Cu, Zn and Mn, and trivalent Fe, Mn and Cr;

R_1, R_2 and R_4 each independently is Y- R_5;

Y is O, S or N R_5R_6;

R_3 is selected from $-CH=CH_2$, $-C(=O)-CH_3$, $-C(=O)-H$, $-CH=NR_7$, $-C(CH_3)=NR_7$, $-CH_2-OR_7$, $-CH_2-SR_7$, $-CH_2-NR_7R'_7$, $-CH(CH_3)-OR_7$, $-CH(CH_3)-SR_7$, $-CH(CH_3)-NR_7R'_7$, $-CH(CH_3)Hal$, $-CH_2-Hal$, $-CH_2-R_7$, $-CH=CR_7R'_7$, $-C(CH_3)=CR_7R'_7$, $-CH=CR_7Hal$, $-C(CH_3)=CR_7Hal$, and $-C\equiv CR_7$;

R_5, R_6, R_7 and R'_7 each is independently H; a straight or branched, saturated or unsaturated, substituted or unsubstituted hydrocarbon chain having from 1 to 25 carbon atoms and optionally interrupted by heteroatoms and/or carbocyclic or heterocyclic moieties; a residue of an amino acid, a peptide or of a protein; and, when Y is O or S, R_5 may further be H or R_8^+;

m is 0 or 1;

R_8^+ is H^+ or a cation;

provided that: (i) at least two of R_5, R_6, R_7 and R'_7 are such a hydrocarbon chain substituted by a negatively charged group or by an acidic group that is converted to a negatively charged group at the physiological pH and/or a residue of an amino acid, a peptide or of a protein; or (ii) at least two of R_1, R_2, and R_4 are OH, SH, $O^- R_8^+$ or $S^- R_8^+$; or (iii) at least one of R_1, R_2, and R_4 is OH, SH, $O^- R_8^+$ or $S^- R_8^+$ and at least one of R_5, R_6, R_7 and R'_7 is such a hydrocarbon chain substituted by a negatively charged group or by an acidic group that is converted to a negatively charged group at the physiological pH and/or a residue of an amino acid, a peptide or of a protein, but excluding the compounds of formula II wherein M is 2H, R_3 is $-C(=O)CH_3$, R_1, R_2 and R_4 are OH, and m is 0 or 1.

The hydrocarbon chain of R_5, R_6, R_7 and R'_7 has 1 to 25, preferably from 1 to 20, more preferably 1 to 5, carbon atoms. It may be straight or branched, saturated or unsaturated, e.g. alkylene or alkenylene chains, optionally interrupted by heteroatoms, such as O, N and/or S, and/or by mono- or poly-carbocyclic, e.g. phenyl, or heterocyclic moieties, and/or substituted by mono- or poly-carbocyclic or heterocyclic moieties, or by one or more functional groups, such as OH, SH, SO_3H, NH_2, $CONH_2$, COOH, PO_3H_2. The negatively charged groups COO^-, COS^-, SO_3^-, and/or PO_3^{2-}, may originate from the functional groups COOH, COSH, SO_3H, and PO_3H_2 groups, respectively, besides the possibility that they may be derived from R_1, R_2, and R_4 being OH or SH, $O^- R_8^+$ or $S^- R_8^+$, namely when a carboxylic or thiocarboxylic group or a carboxylate or thiocarboxylate anion is present at the positions 13^1, 15^1 (m is 0), 15^2 (m is 1), and/or 17^3.

The substituted hydrocarbon chain may also comprise or be the residue of a carbohydrate, e.g., of a mono- or oligosaccharide, of a triglyceride or other lipidic moieties, of a polycarbocyclic ring, e.g., steroids, such as cholesterol, or of a heterocyclic compound, e.g., uracyl and 2,5-dioxo-3,6-dihydroxymethylpiperazine.

In one preferred embodiment, R_5 at any of the positions, but preferably at position 17^3, is the residue of an amino acid, a peptide or a protein. R_5 may be derived from an amino acid or a derivative thereof, such as serine, tyrosine and lysine and derivatives thereof, e.g., L-serine or tyrosine methyl esters, or from a peptide, e.g., seryl serine methyl ester, melanocyte stimulating hormones (MSH), or from a protein, such as immunoglobulin. The amino acid, peptide or protein may be the source of the negatively charged group if they contain a free terminal carboxyl group and/or a residue of an amino acid containing a non-terminal free carboxylic groups, e.g. aspartic or glutamic acid.

As described in US 5,726,169, by conjugation of Bchl with different amino acids, and further conjugation of the Bchl amino acid conjugates with hormones, growth factors or derivatives thereof, or tumor-specific antibodies, or any other cell-specific ligands, suitable site-directed photosensitizers are obtained.

The cation R_8^+ may be a monovalent or divalent cation derived from an alkaline or alkaline earth metal such as K^+, Na^+, Li^+, NH_4^+, Ca^+, more preferably K^+; or is derived from an amine.

In another preferred embodiment, the bacteriochlorophyll derivative of the invention has the formula II wherein:

M represents 2H, divalent Pd, Cu, or Zn or trivalent Mn;

R_1 is $-O^- R_8^+$, $-NH-(CH_2)_n-SO_3^- R_8^+$, or $Y-R_5$ wherein Y is O, S or NH and R_5 is the residue of an amino acid, a peptide or a protein;

R_2 is (C1-C6) alkoxy;

R_3 is $-C(=O)-CH_3$, $-CH=N-(CH_2)_n-SO_3^- R_8^+$; or $-CH_2-NH-(CH_2)_n-SO_3^- R_8^+$;

R_4 is $-NH-(CH_2)_n-SO_3^- R_8^+$; $-NH-(CH_2)_n-COO^- R_8^+$; $-NH-(CH_2)_n-PO_3^{2-}(R_8^+)_2$;

R_8^+ is a monovalent cation; m is 1, and n is an integer from 1 to 10.

In more preferred embodiments of the invention, the bacteriochlorophyll derivative has the formula II and M is Pd.

Thus, in preferred embodiments, in the bacteriochlorophyll derivative of the formula II, M is Pd, R_1 is $-O^- R_8^+$, $-NH-(CH_2)_n-SO_3^- R_8^+$, or $Y-R_5$ wherein R_5 is the residue of a protein, preferably immunoglobulin; R_2 is (C1-C6) alkoxy such as

methoxy, ethoxy, propoxy, butoxy, more preferably methoxy; R_3 is -C(=O)-CH_3, -CH=N-$(CH_2)_n$-SO_3^- R_8^+ ; or –CH_2-NH-$(CH_2)_n$-SO_3^- R_8^+ ; R_4 is–NH-$(CH_2)_n$-SO_3^- R_8^+ ; NH-$(CH_2)_n$-COO^- R_8^+ ; NH-$(CH_2)_n$-PO_3^{2-} $(R_8^+)_2$; R_8^+ is a monovalent cation such as K^+, Na^+, Li^+, NH_4^+, more preferably K^+; m is 1, and n is 2 or 3, more preferably 2.

Examples of bacteriochlorophyll derivatives of the invention having two negatively charged groups at positions 13 and 17 include the compounds identified in the List of Compounds hereinafter as compounds **4**, **5**, **8**, **10**, **11**, **12**, **13**, **14**, **15**. In a most preferred embodiment, the compound of the invention is compound **4**.

Examples of bacteriochlorophyll derivatives of the invention having three negatively charged groups at positions 3, 13 and 17 include the compounds identified in the List of Compounds hereinafter as compounds **9**, **16**. The compound **13** has one negatively charged group at position 13 and a –COOH group as part of the protein molecule at position 17^3, and the compound **15** has one divalent negatively charged group at position 13 and a $–COO^-$ group at position 17^3.

The compounds of the invention can be prepared, for example, by the methods as depicted in Scheme 1 herein. For the preparation of compounds wherein R_5 is the residue of an amino acid, peptide or protein, the methods described in US 5,726,169, particularly the catalytic condensation method, can be used as shown in Scheme 1 for the reaction with the aminosulfonic acids taurine and homotaurine.

Thus, a method for the preparation of compounds of formula II wherein R_1 is -O^- R_8^+; R_2 is $–OCH_3$; R_3 is acetyl; R_4 is a group –NH-$(CH_2)_n$-$SO_3^-$$R_8^+$; R_8^+ is a monovalent cation; m is 1 and n is 1 to 10, comprises: (i) reacting the corresponding M-bacteriopheophorbide of formula I wherein R_1 is OH with an aminosulfonic acid of the formula H_2N-$(CH_2)_n$-SO_3H in a R_8^+-buffer; and (ii) isolating the desired compound of formula II.

For preparation of the compound **4**, the method comprises reacting Pd-bacteriopheophorbide a **3** with taurine of the formula H_2N-$(CH_2)_2$-SO_3H in a K^+ -buffer; and isolating the desired compound.

For preparation of the compound **5**, the method comprises reacting bacteriopheophorbide a **2** with taurine of the formula H_2N-$(CH_2)_2$-SO_3H in a K^+-buffer; and isolating the desired compound.

For preparation of the Cu and Zn compounds **10**, **11**, the method comprises direct insertion of the central metal Cu or Zn atom by reacting the compound **5** with copper acetate or zinc acetate, respectively, while for preparation of the Mn

compound **12**, insertion of the central metal Mn atom is carried out by transmetalation by first reacting the compound **5** with cadmium acetate and then with manganese chloride.

A method for the preparation of compounds of formula II wherein R_1 is $-O^- R_8^+$; R_2 is $-OCH_3$; R_3 is acetyl; R_4 is a group $-NH-(CH_2)_n-COO^- R_8^+$; R_8^+ is a monovalent cation; m is 1 and n is 1 to 10, comprises: (i) reacting the corresponding M-bacteriopheophorbide of formula I wherein R_1 is OH with an aminocarboxylic acid of the formula $H_2N-(CH_2)_n-COOH$ in a R_8^+-buffer; and (ii) isolating the desired compound of formula II.

Thus, for preparation of the compound **14**, the method comprises reacting Pd-bacteriopheophorbide a **3** with β-alanine of the formula $H_2N-(CH_2)_2-COOH$ in a K^+ -buffer; and isolating the desired compound.

A method for the preparation of compounds of formula II wherein R_1 is $-O^- R_8^+$; R_2 is $-OCH_3$; R_3 is acetyl ; R_4 is a group $-NH-(CH_2)_n-PO_3^{2-} (R_8^+)_2$; R_8^+ is a monovalent cation; m is 1 and n is 1 to 10, comprises:(i) reacting the corresponding M-bacterio-pheophorbide of formula I wherein R_1 is OH with an aminophosphonic acid of the formula $H_2N-(CH_2)_n-PO_3H_2$ in a R_8-buffer; and (ii) isolating the desired compound of formula II.

Thus, for preparation of the compound **15**, the method comprises reacting Pd-bacteriopheophorbide a **3** with 3-amino-1-propanephosphonic acid of the formula $H_2N-(CH_2)_3-PO_3H_2$ in a K^+ -buffer; and isolating the desired compound.

For the preparation of compounds having the same negatively charged groups at positions 13 and 17, the corresponding M-bacteriopheophorbide can be reacted with an excess of the reagent such as aminosulfonic, aminocarboxylic or aminophosphonic acid as described above, and isolation of the desired 13,17-disubstituted derivative of formula II, or a different route can be followed as depicted in Scheme 1 herein.

Thus, a method for the preparation of compounds of formula II wherein R_1 and R_4 are each a group $-NH-(CH_2)_n-SO_3^- R_8^+$; R_2 is $-OCH_3$; R_3 is acetyl; R_8^+ is a monovalent cation; m is 1 and n is 1 to 10, comprises: (i) coupling the corresponding M-bacteriopheophorbide of formula I wherein R_1 is OH with N-hydroxy-sulfosuccinimide (sulfo NHS)in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC); (ii) reacting the resulting M-bacteriopheophorbide-17^3-N-hydroxysulfosuccinimide ester with an excess of an aminosulfonic acid of the formula

H_2N-$(CH_2)_n$-SO_3H in a R_8^+-buffer -buffer, thus obtaining an intermediate compound of formula I having a sole negatively charged group at position 17; (iii) reacting this intermediate with an excess of H_2N-$(CH_2)_n$-SO_3H in a R_8^+-buffer; and isolating the desired compound of formula II.

For the preparation of the compound **8**, the reaction is carried out with an excess of homotaurine of the formula H_2N-$(CH_2)_3$-SO_3H.

When the aminosulfonic acid is replaced by aminocarboxylic or aminophosphonic acid, the corresponding carboxylate and phosphonate derivatives are obtained.

The compounds of the invention, also referred herein sometimes by the term "pigments", present sufficient high polarity to be water soluble and injected in aqueous solutions with no added surfactants. In one embodiment, for the preferred sulfonated-Pd-Bchl compound **4**, also biodistribution and pharmacokinetics are shown and, based thereon, it is assumed that this and the other derivatives of the invention remain in the circulation, and for a very short time. Therefore they are good sensitizers for vascular-targeting PDT. Treatment of M2R melanotic melanoma and HT-29 human colon carcinoma xenografts in mice shown herein, demonstrate the selective effect of the pigment on the tumor vasculature. The suggested protocol with sulfonated-Pd-Bchl **4** considered the short clearance time of the drug. On the ground of their selective effect on the tumor vasculature, these compounds can be used for tumor as well as age-related macular degeneration and other tissues abnormalities that depend on neovascularization.

Thus, in another aspect, the present invention provides a pharmaceutical composition comprising a bacteriochlorophyll derivative of the invention and a pharmaceutically acceptable carrier.

In a preferred embodiment, the pharmaceutical composition comprises a bacteriochlorophyll derivative of formula I or II herein, more preferably a sulfonated derivative of formula II, most preferably the compound **4**.

The new bacteriochlorophyll compounds of the invention have similar optical absorption and photophysical characteristics as the respective Bchls and, therefore, once residing within the treated tissue, they are expected to be efficient photodynamic agents. They can thus be useful as photosensitizers as therapeutic and diagnostic agents, for example for treatment of several cancer types such as, but not limited to, melanoma, prostate, brain, colon, ovarian, breast, skin, lung, esophagus and bladder

cancers and other hormone-sensitive tumors, as well as for treatment of age-related macular degeneration (AMD), and for killing cells, viruses, fungi and bacteria in samples and living tissues as well known in the art of PDT and other photosensitizer applications.

The new water-soluble Bchl derivatives of the invention are useful, for example, in sensitizing neoplastic cells or other abnormal tissue to destruction by irradiation either *in vivo* or *ex vivo* using light of appropriate wavelength. It is believed that the energy of photoactivation is transferred to endogenous oxygen to convert it to singlet oxygen, and/or other reactive oxygen species (ROS) such as superoxide and hydroxyl radicals, which are considered to be responsible for the cytotoxic effect. In addition, the photoactivated forms of the bacteriochlorophylls fluoresce, which fluorescence can aid in localizing tumors or other sites to which the bacteriochlorophylls are administered.

Examples of indications, known in the art, that can be treated with the bacteriochlorophyll derivatives of the present invention, include destruction of tumor tissue in solid tumors and dissolution of plaques in blood vessels (see, e.g., US Patent No. 4,512,762). Particularly, these derivatives are suitable for vascular-targeted PDT because of their minimal retention in the circulation and because they are taken-up only minimally by non-circulating tissues such as skin and muscle. Thus, these compounds enable reactive oxygen species (ROS) generation limited to the interior vessels upon excitation and, thereby, cause selective response of abnormal vessels such as those present in tumors and age-related macular degeneration. In addition, the bacteriochlorophyll derivatives are useful for selective destruction in treatment of topical conditions such as acne, athlete's foot, warts, papilloma, and psoriasis, for treatment of benign prostate hypertrophy and for sterilization of biological products such as blood for transfusion, by destruction of infectious agents.

The bacteriochlorophyll derivatives of the present invention are formulated into final pharmaceutical compositions for administration to the patient or applied to an *in vitro* target using techniques well-known in the art, for example, as summarized in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Penna., latest edition. The compositions can be administered systemically, in particular by injection, or can be used topically.

For diagnosis, the bacteriochlorophyll derivatives may be used alone or may be labeled with a radioisotope or other detecting means as known in the art.

The amount of bacteriochlorophyll derivative to be administered will be according to the experience accumulated with other porphyrins used in PDT, and will vary depending on the choice of the derivative used as active ingredient, the condition to be treated, the mode of administration, the age and condition of the patient, and the judgement of the physician.

The wavelenght of irradiating light is preferably chosen to match the maximum absorbance of the bacteriochlorophyll photosensitizer. The suitable wavelength for any of the compounds can readily be determined from its absorption spectrum.

In addition to *in vivo* use, the bacteriochlorophyll derivatives of the invention can be used in the treatment of materials *in vitro* to kill harmful viruses or infectious agents, such as harmful bacteria. For example, blood and blood plasma to be used for future transfusion can be treated with a compound of the invention and irradiated to effect sterilization.

The conjugation of proteins, e.g., hormones, growth factors or their derivatives, antibodies, peptides that bind specifically to target cells receptors, and of cell nutrients, e.g. tyrosine, to the Bchl moiety is meant to increase their retention in tumor and treated sites. Increasing the red shift allows for a greater depth of penetration, while keeping the ubiquity of the natural system. Replacement of the Mg by other metals is meant to optimize the intrinsic and metabolic stability of the Bchl moiety and its intersystem crossing to the excited triplet state, and also opens the possibility for new diagnostic procedures.

Tumor-specific antibodies and peptides that have high affinity to neoendothelial cells will preferentially target the Bchl moieties to the tumor or treated site, while hormones and cell nutrients may also be taken up by the normal non-transformed counterparts. However, the cells selected as targets to hormones and cell nutrients, such as melanocytes and neoendothelial cells are scattered among other cells under normal conditions and when transformed into malignant cells, cluster into solid tumors. As a result, the concentration of the photosensitizer in the vascular and/or cellular compartments of the malignant tissue is expected to increase dramatically relative to its concentration in the normal tissue, where cells are more dispersed, assuring amplification of the PDT effect in the tumor site. This enables effective use of light doses, lower than the damaging threshold of the normal tissue, thus reducing the need for spatially well-defined irradiation. In addition, having very

strong fluorescence, the site-directed Bchl can be used for fluorescence labeling of the tumor site(s) or other targets.

In one most preferred embodiment of the present invention, the target for treatment with the sensitizers of the invention are abnormal blood vessels, particularly blood vessels of solid tumors and age-related macular degeneration, due to the inherent difference of sensitivity of normal and abnormal blood vessels to the suggested PDT protocols described herein.

The pharmaceutical compositions of the invention will be administered to the patient by standard procedures used in PDT. The amount of compound to be administered and the route of administration will be determined according to the kind of tumor, stage of the disease, age and health conditions of the patient, but will be much lower than currently used dosage of Photofrin II of about 20-40 mg HPD/kg body weight. The preferable routes of administration are intravenous or direct injection into the solid tumor of the aqueous solution of the active compound comprising conventional pharmaceutically acceptable carriers and additives, and topical treatment of skin tumors with suitable topical compositions.

The invention further relates to a method of photodynamic therapy, which comprises administering to an individual in need an effective amount of a Bchl derivative of the invention, followed by local irradiation.

In one embodiment, the PDT method of the invention is used for treatment of cancer and comprises administering to a patient afflicted with a solid tumor cancer a pharmaceutical composition comprising a Bchl derivative according to the invention, and then irradiating the tumor site with strong light sources at 670-780 nm.

The Bchl derivatives of the invention are also useful for photodestruction of normal or malignant animal cells as well as of microorganisms in culture, enabling selective photodestruction of certain types of cells in culture or infective agents; for targeting of the porphyrin moiety to selected cells by attachment to specific polypeptides, such as hormones or other receptor ligands, to cell- or tissue-specific antibodies or to other ligands, e.g., lectins; for fluorescent labeling/tagging of molecules for analytical purposes in laboratory, diagnostic and industrial applications; and for fluorescent labeling of animal cells or microorganisms or particles for laboratory, diagnostic or industrial applications. They can replace several of the currently used fluorescence tags, such as fluorescein isothiocyanate (FITC) or

phycoerythrine, due to their superior extinction coefficients and higher fluorescence yield.

For diagnostic purposes, the Bchl derivatives of the invention can be radioactively-labeled by standard procedures, e.g., with ^{67}Ga, ^{111}In, $^{201}T1$, ^{99}mTc, and the radioactive diagnostic agent is administered to the patient, preferably by i.v.-injection. After some hours, the locus of the cancer may be imaged by standard procedures.

The invention further provides the use of the Bchl derivatives of the invention for *ex-vivo* or *in vitro* killing of cells or infectious agents such as bacteria, viruses, parasites and fungi in a biological product, e.g. blood, which comprises treating the infected sample with the compound of the invention followed by illumination of the sample.

The invention will now be illustrated by the following non-limitative Examples.

EXAMPLES

For convenience and better understanding, the section of the Examples is divided into two subsections: (I) the Chemical Section, describing the synthesis of the water-soluble derivatives and intermediates **4-16**, and (II) the Biological Section, describing the biological activity of the new Bchl derivatives.

I CHEMICAL SECTION

In the Examples herein, the derivatives of the invention (**4-5, 8-9, and 10-16**) and the intermediates (**1-3, and 6,7**) will be presented by their respective Arabic numbers in bold and underlined according to the following List of Compounds. The corresponding formulas appear in the Scheme at the end of the specification, just before the claims.

List of Compounds

1. Bacteriochlorophyll a

2. Bacteriopheophorbide a

3. Pd-Bacteriopheophorbide a

4. Palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt [Example 1]

5. 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt [Example 2]

6. Palladium bacteriopheophorbide a 17^3–(3-sulfo-1-oxysuccinimide) ester sodium salt [Example 6]

7. Palladium Bacteriopheophorbide a 17^3-(3-sulfopropyl)amide potassium salt [Example 7]

8. Palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide dipotassium salt [Example 8]

9. Palladium 3^1-(3-sulfopropylimino)-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide tripotassium salt [Example 9]

10. Copper(II) 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt [Example 3]

11. Zinc 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl) amide dipotassium salt [Example 4]

12. Manganese(III) 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt [Example 5]

13. Palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide, 17^3-(N-immunoglobulin G)amide potassium salt] [Example 10]

14. Palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-carboxyethyl)amide dipotassium salt [Example 11]

15. Palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(3-phosphopropyl)amide tripotassium salt [Example 12]

16. Palladium 3^1-(3-sulfopropylamino)-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide tripotassium salt [Example 13]

Materials and methods

Bchl a (1) was extracted and purified from lyophilized cells of *Rhodovolum Sulfidophilum* as previously described (WO 00/33833).

Palladium bacteriopheophorbide (Pd-Bpheid, 2) was either prepared as previously described (WO 00/33833) or it was obtained from Steba Biotech Ltd. through Negma-Lerads, France.

3-Amino-1-propane sulfonic acid (homotaurine) and 3-amino-1-propane phosphonic acid were purchased from Aldrich (USA), and 2-aminoethane sulfonic acid (taurine) and 3-aminopropionic acid (β-alanine) were purchased from Sigma (USA), N-

hydroxy-sulfosuccinimide (sulfo-NHS) was purchased from Pierce (USA), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) was purchased from Fluka (Switzerland).

Chemicals and solvents of analytical grade were generally used except when performing HPLC, where HPLC-grade solvents were applied.

TLC: silica plates (Kieselgel-60, Merck, Germany); chloroform-methanol (4:1, v/v).

^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on Avance DPX 250 instrument (Bruker, France) and reported in ppm (δ) downfield from tetramethylsilane with residual solvent peaks as the internal standards.

The extinction coefficients of the Pd-derivatives were determined by correlating the Pd concentration (using flame photometry with $PdCl_2$ as a standard) with the optical density of the examined solution at the particular wavelength.

Electrospray ionization mass spectra (ESI-MS) were recorded on a platform LCZ spectrometer (Micromass, England).

Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) was performed for determination of Pd concentrations using an ELAN-6000 instrument (Perkin Elmer, CT).

Optical absorption of the different complexes was recorded with Genesis-2 (Milton Roy, England) and V-570 (JASCO, Japan) spectrophotometers.

HPLC was performed using an LC-900 instrument (JASCO, Japan) equipped with a UV-915 diode-array detector.

CHEMICAL EXAMPLES

Example 1. Palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt (Compound 4)

Nine hundred and thirty five (935) mg of Pd-Bpheid (3) were dissolved in a 1 L round bottom flask with 120 ml of DMSO while stirring under Argon (bubbled in the solution). Taurine (1288 mg) was dissolved in 40 ml of 1M K_2HPO_4 buffer, and the pH of the solution was adjusted to 8.2 (with HCl). This aqueous solution was added into the DMSO solution while stirring, and the Argon was bubbled in the solution for another 20 minutes. Then the reaction mixture was evaporated at 30°C for 3.5 hours under ~2 mbar and then for another 2 hours at 37°C to a complete dryness.

The dry solids were dissolved in 300 ml of MeOH and the colored solution was filtered through cotton wool to get rid of buffer salts and taurine excess.

The progress of the reaction was determined by TLC (R_f of unreacted Pd-Bpheid is 0.8-0.85 and of the reaction (aminolysis) product is 0.08-0.1) and by following the optical absorption spectrum of the reaction mixture after liophylization and resolubilization in MeOH. The absorption spectrum was characterized by a Q_y transition shift from 756 nm (for Pd-Bpheid) to 747 nm (for the product **4**) and by Q_x shift from 534 nm of Pd-Bpheid to 519 nm (of the product **4**). The MeOH was evaporated and the product **4** was purified by HPLC with ODS-A 250X20 S10P μm column (YMC, Japan). Solvent A: 95% 0.005 M phosphate buffer, pH 8.0 and 5% MeOH. Solvent B: 100% MeOH. The dry solid was dissolved in 42 ml of distilled water and injected in portions of 1.5 ml each .

The elution profile is described in Table 1. The product **4** (Scheme 1, see below) was eluted and collected at ~ 9-11 minutes. The main impurities, collected after at 4-7 min (ca 5-10%), corresponded to byproduct(s) with the proposed structure **7**. Peaks at 22-25 min (ca 2-5%) possibly corresponded to the iso-form of the main product **4** and untreated Pd-Bpheid residues.

Table 1. Gradient profile of purification of compound 4

Time (min)	Flow(ml/min)	A%	B%
0	12	55	45
14	12	30	70
14.1	6	30	70
16	6	0	100
18	6	0	100
24	6	55	45
29	6	55	45
30	0.5	55	45

The solvent (aqueous methanol) was evaporated under reduced pressure. Then, the purified product **4**]was re-dissolved in ~150 ml MeOH and filtered through

cotton wool. The solvent was evaporated again and the solid pigment 4 was stored under Ar in the dark at –20^{0}C. The reaction yield: ~90% (by weight, relative to 3).

The structure of product 4 was confirmed by electrospray mass spectroscopy. (ESI-MS, negative mode, Fig.2), (peaks at 875 (M^--K-H), 859 (M^--2K-H+Na), 837 (M^--2K), 805 (M2K-H-OMe), 719) and ^{1}H-NMR spectrum (Fig. 4 in MeOH-d_4). Table 4 provides the shifts (in ppm units) of the major NMR peaks.

Optical absorption (UV-VIS) spectrum (MeOH): λ, 747 (1.00), 516 (0.13), 384 (0.41), 330 (0.50); ε_{747} (MeOH) is 1.2 x 10^5 mol^{-1} cm^{-1}.

NMR (MeOH-d_4): 9.38 (5-H, s), 8.78 (10-H, s), 8.59 (20-H, s), 5.31 and 4.95 (15^1-CH_2, dd), 4.2-4.4 (7,8,17,18-H, m), 3.88 (15^3-Me, s), 3.52 (2^1-Me, s), 3.19 (12^1-Me, s), 3.09 (3^2-Me, s), 1.92-2.41, 1.60-1.75 (17^1, 17^2-CH_2, m), 2.19 (8^1-CH_2, m), 1.93 (7^1-Me, d), 1.61 (18^1-Me, d), 1.09 (8^2-Me, t), 3.62, 3.05 (CH_2's of taurine).

Octanol/water partition ratio is 40:60.

Example 2. Preparation of 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt (Compound 5)

One hundred and sixty (160) mg of taurine were dissolved in 5 ml of 1M K_2HPO_4 buffer, and the pH of the solution was adjusted to 8.2. This solution was added to 120 mg of compound 2 dissolved in 15 ml of DMSO, and the reaction and following purification were analogous to those described in previous Example.

Absorption spectrum (MeOH): λ, 750 (1.00), 519 (0.30), 354 (1.18) nm.

ESI-MS (-): 734 (M^--2K).

NMR (MeOH-d_4): 9.31 (5-H, s), 8.88 (10-H, s), 8.69 (20-H, s), 5.45 and 5.25 (15^1-CH_2, dd), 4.35 (7,18-H, m), 4.06 (8,17-H, m), 4.20 and 3.61 (2-CH_2, m of taurine), 3.83 (15^3-Me, s), 3.63 (2^1-Me, s), 3.52 (3-CH_2, m of taurine), 3.33 (12^1-Me, s), 3.23 (3^2-Me, s), 2.47 and 2.16 (17^1-CH_2, m), 2.32 and 2.16 (8^1-CH_2, m), 2.12 and 1.65 (17^2-CH_2, m), 1.91 (7^1-Me, d), 1.66 (18^1-Me, d), 1.07 (8^2-Me, t).

Octanol/water partition ratio is 60:40.

Example 3. Preparation of copper(II) 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt (Compound 10)

Fifty (50) mg of compound 5 of Example 2 and 35 mg of copper (II) acetate were dissolved in 40 ml of methanol, and argon was bubbled into solution for 10

minutes. Then 500 mg of palmitoyl ascorbate was added, and the solution was stirred for 30 min. The absorption spectrum was characterized by a Q_y transition shift from 750 nm (for **5**) to 768 nm (for the product **10**) and by Q_x shift from 519 nm of **5** to 537 nm (of the product **10**). Then the reaction mixture was evaporated, re-dissolved in acetone and filtered through cotton wool to get rid of acetate salt excess. The acetone was evaporated and the product was additionally purified by HPLC at the conditions mentioned above with the elution profile, described in Table 2.

The solvent (aqueous methanol) was evaporated under reduced pressure. Then, the purified pigment **10** was re-dissolved in methanol and filtered through cotton wool. The solvent was evaporated again and the solid pigment **10** was stored under Ar in the dark at -20^0C. Reaction yield: ~90%.

Table 2. Gradient profile of purification of compound 10

Time (min)	Flow(ml/min)	A%	B%
0	12	58	42
14	12	45	55
14.1	6	45	55
16	6	0	100
18	6	0	100
24	6	58	42
29	6	58	42
30	0.5	58	42

Absorption spectrum (MeOH): λ, 768 (1.00), 537 (0.22), 387 (0.71) and 342 (0.79) nm.

ESI-MS (-): 795 (M^--2K).

Octanol/water partition ratio is 40:60.

Example 4. Preparation of zinc 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt (Compound 11)

Zn insertion into compound **5** was carried out with Zn acetate in acetic acid as previously described (US Patent No. 5,726,169). Final purification was carried out by HPLC in the same conditions as for compound **5** in Example 2 above.

Absorption spectrum (MeOH): λ, 762 (1.00), 558 (0.26), 390 (0.62) and 355 (0.84) nm.

Octanol/water partition ratio is 50:50.

Example 5. Preparation of manganese(III) 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt (Compound 12)

Mn insertion into compound 5 was carried out with Zn acetate in acetic acid as previously described (WO 97/19081; US 6,333,319) with some modifications. Thus, fifty (50) mg of compound 5 in 10 ml of DMF were stirred with 220 mg of cadmium acetate and heated under argon atmosphere at 110°C about 15 min (Cd-complex formation is monitored by shifting Q_x transition absorption band from 519 to 585 nm in acetone). Then the reaction mixture was cooled and evaporated. The dry residue was re-dissolved in 15 ml of acetone and stirred with manganese (II) chloride to form the Mn(III)-product 12. The product formation is monitored by shifting Q_x transition band from 585 to 600 nm and Q_y transition band from 768 to 828 nm in acetone. The acetone was evaporated and the product 12 was additionally purified by HPLC in the conditions mentioned in Example 2 above with the elution profile described in Table 3 below where the solvent system consists of: A - 5% aqueous methanol, B - methanol.

Table 3. Gradient profile of purification of compound 12

Time (min)	Flow(ml/min)	A%	B%
0	8	95	5
14	8	55	45
14.1	8	55	45
16	8	0	100
18	8	0	100
24	8	95	5
29	8	95	5
30	0.5	95	5

The solvent (aqueous methanol) was evaporated under reduced pressure and the solid pigment 12 was stored under Ar in the dark at –20°C.

Absorption spectrum (MeOH): λ, 828 (1.00), 588 (0.32) and 372 (0.80) nm. Octanol/water partition ratio is 5:95.

Example 6. Preparation of palladium bacteriopheophorbide a 17^3–(3-sulfo-1-oxy-succinimide)ester sodium salt (Compound 6)

Fifty (50) mg of Pd-Bpheid (compound 2), 80 mg of N-hydroxysulfosuccinimide (sulfoNHS) and 65 mg of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) were mixed in 7 ml of dry DMSO for overnight at room temperature. Then the solvent was evacuated under reduced pressure. The dry residue was re-dissolved in chloroform (ca. 50 ml), filtered from insoluble material, and evaporated. The conversion was ab. 95% (TLC). The product 6 was used later on without further chromatographic purification. ESI-MS (-): 890 (M^--Na).

NMR ($CDCl_3$): 9.19 (5-H, s), 8.49 (10-H, s), 8.46 (20-H, s), 5.82 (13^2-H, s), 4.04-4.38 (7,8,17,18-H, m), 3.85 (13^4-Me, s), 3.47 (2^1-Me, s), 3.37 (12^1-Me, s), 3.09 (3^2-Me, s), 1.77 (7^1-Me, d), 1.70 (18^1-Me, d), 1.10 (8^2-Me, t), 4.05 (CH_2 of sNHS), 3.45 (CH of s NHS).

Example 7. Preparation of palladium bacteriopheophorbide a 17^3-(3-sulfopropyl) amide potassium salt (Compound 7)

Ten (10) mg of compound 6 in 1 ml of DMSO was mixed with 20 mg of homotaurine (3-amino-1-propane-sulfonic acid) in 1 ml of 0.1 M K-phosphate buffer, pH 8.0 for overnight. Then the reaction mixture was partitioned in chloroform/water. The organic layer was dried over anhydrous sodium sulfate and evaporated. The dry residue was re-dissolved in chloroform-methanol (19:1) and applied to a chromatographic column with silica. The product 7 was obtained with chloroform-methanol (4:1) elution. The yield was about 80-90%.

ESI-MS (-): 834 (M-K) m/z.

NMR (MeOH-d_4): 9.16 (5-H, s), 8.71 (10-H, s), 8.60 (20-H, s), 6.05 (13^2-H, s), 4.51, 4.39, 4.11, 3.98 (7,8,17,18-H, all m), 3.92 (13^4-Me, s), 3.48 (2^1-Me, s), 3.36 (12^1-Me, s), 3.09 (3^2-Me, s), 2.02-2.42 (17^1 and 17^2-CH_2, m), 2.15 (8^1-CH_2, q), 1.81 (7^1-Me, d), 1.72 (18^1-Me, d), 1.05 (8^2-Me, t), 3.04, 2.68, and 2.32 (CH_2's of homotaurine, m).

Example 8. Preparation of palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodo-bacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide dipotassium salt (Compound 8)

Ten (10) mg of compound 6 or 7 were dissolved in 3 ml of DMSO, mixed with 100 mg of homotaurine in 1 ml of 0.5 M K-phosphate buffer, pH 8.2, and incubated overnight at room temperature. The solvent was then evacuated under reduced pressure as described above, and the product 8 was purified on HPLC. Yield: 83%.

Absorption spectrum (MeOH): 747 (1.00), 516 (0.13), 384 (0.41), 330 (0.50), ε_{747} $=1.3\times10^5$ $mol^{-1}cm^{-1}$.

ESI-MS(-):1011 (M^--K), 994 (M^--2K+Na),972 (M^--2K), 775 (M^--2K-CO_2Me-homotaurine $NHCH_2CH_2CH_2SO_3$), 486 ([M-2K]/2)

NMR (MeOH-d_4): 9.35 (5-H, s), 8.75 (10-H, s), 8.60 (20-H, s), 5.28 and 4.98 (15^1-CH_2, dd), 4.38, 4.32, 4.22, 4.15 (7,8,17,18-H, all m), 3.85 (15^3-Me, s), 3.51 (2^1-Me, s), 3.18 (12^1-Me, s), 3.10 (3^2-Me, s 2.12-2.41 (17^1-CH_2, m), 2.15-2.34 (8^1-CH_2, m), 1.76-2.02 (17^2-CH_2, m), 1.89 (7^1-Me, d), 1.61 (18^1-Me, d), 1.07 (8^2-Me, t). 3.82, 3.70, 3.20, 3.10, 2.78, 2.32, 1.90 (CH_2's of homotaurine at C-13^1 and C-17^3)

Example 9. Palladium 3^1-(3-sulfopropylimino)-15-methoxycarbonylmethyl-Rhodo-bacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide tripotassium salt (Compound 9)

Compound 9 was obtained from HPLC as a minor product during synthesis of 8.

Absorption spectrum (MeOH): 729 (1.00), 502 (0.10), 380 (0.69), 328 (0.57).

ESI-MS (30.4.2000): 1171 (M-K+H), 1153 (M^--2K-H+Na), 1131 (M-2K), 566 ([M-K]/2), 364 ([M-3K]/3).

NMR (MeOH-d_4): 8.71 (1H), 8.63 (1.5H), 8.23 (0.5H) (5-, 10- and 20-H, all-m), 5.30 and 4.88 (15^1-CH_2, dd), 4.43 and 4.25 (7,8,17,18-H, m), 3.85 (15^3-Me, s), 3.31 (2^1-Me, s), 3.22 (12^1-Me, s), 3.17 (3^2-Me, m), 1.89-2.44 (17^1 and 17^2-CH_2, m), 2.25 (8^1-CH_2, m), 1.91 (7^1-Me, s), 1.64 (18^1-Me, s), 1.08 (8^2-Me, t), 4.12, 3.56, 3.22, 3.16, 2.80 and 2.68 (CH_2's of homotaurine).

Example 10. Palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-sulfoethyl)amide, 17^3-(N-immunoglobulin G)amide potassium salt (Compound 13)

Ten (10) mg of compound 4 were reacted with 20 mg of sulfo-NHS and 15 mg of EDC in 1 ml of dry DMSO for 1 hour at room temperature, then rabbit IgG (0.6 mg) in PBS (2.5 ml) was added, and the mixture was further incubated overnight at room temperature. The mixture was evaporated to dryness, then re-dissolved in 1 ml of PBS and loaded on Sephadex G-25 column equilibrated with PBS. A colored band was eluted with 4-5 ml of PBS. The pigment/protein ratio in the obtained conjugate 13 was determined by optical density at 753 and 280 nm, respectively, and varied between 0.5/1 to 1/1 of pigment 13/protein.

Example 11. Preparation of palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(2-carboxyethyl)amide dipotassium salt (Compound 14)

The preparation and purification of the title compound 14 were carried out as described in Example 2, by reaction of compound 2 with 3-aminopropionic acid (β-alanine) (150 mg) instead of taurine. Yield: 85%.

Example 12. Preparation of palladium 3^1-oxo-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1-(3-phosphopropyl)amide tripotassium salt (Compound 15)

The preparation and purification of the title compound 15 were carried out as described in Example 2, by reaction of compound 2 with 3-amino-1-propanephosphonic acid (180 mg) instead of taurine. Yield: 68%.

Example 13. Palladium 3^1-(3-sulfopropylamino)-15-methoxycarbonylmethyl-Rhodobacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide tripotassium salt (Compound 16)

For reduction of the imine group in 3^1-(3-sulfopropylimino) to the correspondent 3^1-(3-sulfopropylamino) group, compound 9 (8 mg) was reacted by stirring with sodium cyanoborohydride (15 mg) in 5 ml of methanol overnight at room temperature. Then the reaction mixture was treated with 0.05 M HCl (5 ml),

neutralized with 0.01 M KOH, and evaporated. The title product **16** was purified using HPLC conditions as described in Example 2. Yield: 80-90%.

II BIOLOGICAL SECTION

Materials and Methods

***In Vitro* Studies**

(i) Cell Culture. M2R mouse melanoma , H5V mouse endothelial and C6 rat glioma cells were cultured as monolayers in Dulbecco's modified Eagle's medium (DMEM)/F12 containing 25 mM HEPES, pH 7.4, 10% fetal bovine serum (FBS), glutamine (2 mM), penicillin (0.06 mg/ml), and streptomycin (0.1 mg/ml) (hereinafter referred to as the "Culture Medium"). Cells were grown at 37^0C in an 8% CO_2-humidified atmosphere.

(ii) Phototoxicity Assay. To determine the photodynamic efficacy, cells were preincubated with increasing concentrations of the pigments in the dark for the times and conditions as indicated for the individual experiments. Unbound sensitizer was removed by washing the cells once with culture medium, and the plates were illuminated at room temperature from the bottom (λ>650 nm, 12 J/cm^2). The light source was a 100W Halogen lamp (Osram, Germany) equipped with a 4-cm water filter. The cultures were placed in the culture incubator and cell survival was determined 24 h after illumination, by Neutral Red viability assay. Three kinds of controls were used: (i) light control: cells illuminated in the absence of pigments; (ii) dark control: cells treated with pigments but kept in the dark; and (iii) untreated cells that were kept in the dark.

***In Vivo* Studies**

(iii) Tumor Implantation. M2R or C6 cells (2×10^6) were implanted subcutaneously on the back of the mice; tumors developed to the treatment size (6-8 mm) within 2-3 weeks.

(iv) Preparation of Sensitizer. Stock solutions of the compounds of the invention were prepared prior to use by dissolving the dry pigment directly in PBS to the desired concentration for injection.

(v) Biodistribution and Pharmacokinetics. Pigment **4** of the invention (6 mg/kg body) was injected to CD1 nude mice via tail vein. Mice were sacrificed at the indicated times, and samples of the indicated organs or tissues were placed and weighed in pre-weighted vials and immediately frozen on dry ice. For examination, each sample was thawed and homogenized (1:10 w/v) in double-distilled water. Aliquots of the homogenate (0.5 ml) were lyophilized in Eppendorff test tubes. Then 0.2 ml of HNO_3 (70%, TraceSelect, Fluka) was added to each dry sample, incubated for 1 h at 90°C and diluted in double- distilled water to 10 ml. Palladium concentrations were determined by ICP-MS. Background was determined for each organ/tissue on identical samples taken from untreated mice, and values were subtracted accordingly.

(vi) PDT Protocol. The M2R tumor-bearing mice were anesthetized and the pigment was injected intravenously (i.v.) via the tail vein. The tumors were immediately illuminated transcutaneously for 5 min by 755 nm diode laser (CeramOptec, Germany) with light dose of either 30J/cm2 (100mW/cm2), 39J/cm2 (130mW/cm2) or 45J/cm2 (150mW/cm2). After the treatment, the mice were returned to the cage. In the dark control group, the mice were injected i.v. with sensitizer and placed in the dark cage for 24 h. In the light control group, the mice were illuminated with 45J/cm^2.

(vii) Vascular Shutdown and Permeability. Mice bearing C6 glioma tumor xenografts were treated with pigment **4** (9 mg/kg) and light (100 mW/cm^2 for 5 min). Immediately after treatment, Evans Blue (EB; 1% in PBS) was injected (0.5 ml, i.p.). Mice were photographed at 3 and 24 hours after treatment. The mice were sacrificed 24 hours after treatment and skin flap was made for each mouse and photographed. Then the tumor was removed with the skin above it, frozen for 1 hour at –20°C, and then axial slice was made and the slice was photographed. Control mice were injected with Evans Blue at the same time as the treated mice, and the protocol was continued as described above for all the mice together.

Example 14. Cytophotoxicity of the sulfonated bacteriochlorophyll derivatives against tumor cell cultures

The phototoxicity of compounds **4** and **8** was determined as described in (ii) above in M2R mouse melanoma and H5V mouse endothelial cells. Cells were preincubated with increasing concentrations of the compound for 4 hours, washed and illuminated or kept in the dark.

The results are shown in **Figs. 1A-1B** for the bi-sulfonated compound **8** in H5V and MR2 cells, respectively, and in Figs. 2A-2B for the mono-sulfonated compound **4** (comparison) in H5V and MR2 cells, respectively. As can be seen, the phototoxicity of both pigments **4** and **8** is concentration- and light-dependent, without any dark toxicity in the tested range. The LD_{50} of both pigments is the same (~2 μM), and is similar in both cell lines.

The phototoxicity of the sulfonated pigments **5 and 11** was determined on M2R mouse melanoma cells. As can be seen in **Figs. 3 and 4,** the phototoxicity of pigments **5** and **11** is concentration- and light-dependent, and the LD_{50} of both pigments is the same (~5μM). There is no dark toxicity within the tested range.

Example 15. Pharmacokinetics and biodistribution of compound 4

The first step before testing the phototoxicity of **4** toward PDT of solid melanoma xenografts was to determine the pigment's pharmacokinetics and biodistribution *in vivo* as described in section (vi) above. As can be seen in **Fig. 5,** about 90% of the pigment **4** cleared within the first 10-min after i.v. injection with a monophasic kinetic pattern with a $t_{0.5}$ of 1.65 min (**Table 4**). The fast clearance of **4** from the blood may imply that only a small fraction (if at all) is bound to the plasma components, otherwise clearance might have been slower.

Table 4 . Pharmacokinetic parameters of **4** in mice blood.

Parameter	
Equation	y=1.64+90.6e(-0.42t)
$T_{0.5}$ (min)	1.65
K_{el} (min^{-1})	0.42
Vd (ml)	2.12
CL (ml/min)	0.89

K_{el} – rate of elimination; Vd – volume of distribution; CL – clearance.

The biodistribution of the compound 4 shows that, in most of the examined organs of the mouse, the pigment levels are high immediately after injection and drop to almost background levels within 20-30 min, similar to their clearance rates from the blood (**Fig. 6**). These results probably represent the pigment level in the blood trapped in the organ's vasculature as seen in spleen, lung, and heart. Furthermore, the results also suggest that pigment diffusion into the organs is negligible. The pigment 4 clears rapidly from the mouse body, and within 30 min after injection it is in background levels in all tissues. The clearance rate of 4 from the mouse body is much faster than Pd-Bpheid (3), which reaches background levels only 48 hours after injection (not shown).

Example 16. Photodynamic treatment of M2R melanoma xenografts in CD1 nude mice with sulfonated pigment 4

Based upon the pharmacokinetic results of Example 15 above, the treatment protocol for compound 4 was set to 5-min illumination immediately after pigment injection. In these experiments (see section (vii) above), a dedicated medical laser matched to the peak absorption of 4 (CeramOptec, Germany, 755 nm) was used. In order to determine the optimal drug/light protocol, mice were treated with drug dose of 6 mg/kg and increasing the light intensity (**Fig. 7**). As can be seen in the Kaplan-Meier survival curve, increasing the light intensity improves the mice cure rate from 43% to 60% with 30 and 45 J/cm^2, respectively. When the drug dose was elevated to 9 mg/kg with light intensity of 30 J/cm^2, there was a significant increase in the mice survival to 70% (**Fig. 7**). No dark toxicity was seen in animals treated with 6 or 9 mg/kg and kept in the dark.

Example 17. Selective effect of photodynamic treatment with compound 4

This experiment was carried out as described in section (vii) above. **Fig. 8** illustrates the effect of photodynamic treatment on blood perfusion in C6 xenografts implanted in mice (a, e). Treated animal that was administrated with Evans-Blue immediately after PDT showed edema and enhanced vascular leakage of EB into the interstitium as demonstrated by the blue color (due to albumin-Evans Blue leakage) in the illuminated area when compared to the non-illuminated area in the same animal an to untreated animal (b, f). Twenty-four hours later, it can be seen that in the treated mice, the tumor surrounding is heavily colored blue (edema; c), while the tumor

remains white (no EB color) due to vascular shutdown that occurred immediately after PDT (d). The muscle tissue under the tumor as well as the skin above and around the tumor (but within the treated area) is blue, indicating that no vascular shutdown took place (c, d). In the untreated animal, the tumor is colored blue like other tissues (g, h). The selective enclosure of new vessels in the tumor indicates that the compounds of the invention can be used for selective treatment of abnormal vasculature as in age-related macular degeneration (AMD).

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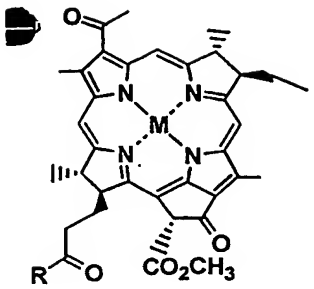
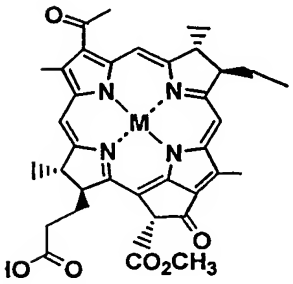
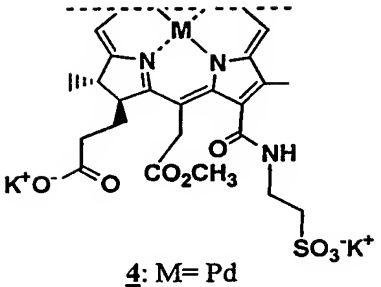


1: M=Mg, R=phytyl or geranylgeranyl (Bchl a)
R=Seryl (Bchl-Ser)



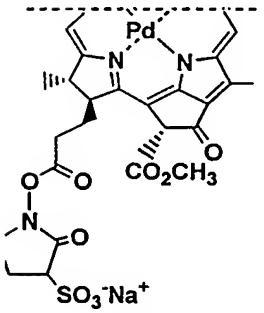
2: M= 2H
3: M= Pd

$H_2N\text{-}(CH_2)_n\text{-}SO_3H$
DMSO/K-phosphate buffer



4: M= Pd
5: M= 2H
10: M= Cu^{II}
11: M= Zn
12: M= Mn^{III}

sulfo NHS
EDC
DMSO



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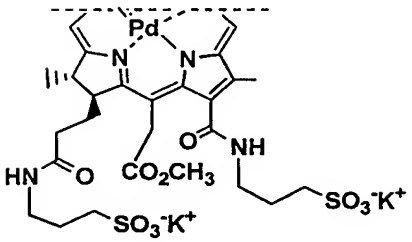
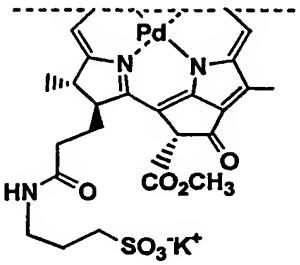
$H_2N\text{-}(CH_2)_3\text{-}SO_3H$
excess
DMSO/K-phosphate buffer



8

$H_2N\text{-}(CH_2)_3\text{-}SO_3H$
DMSO/K-phosphate buffer



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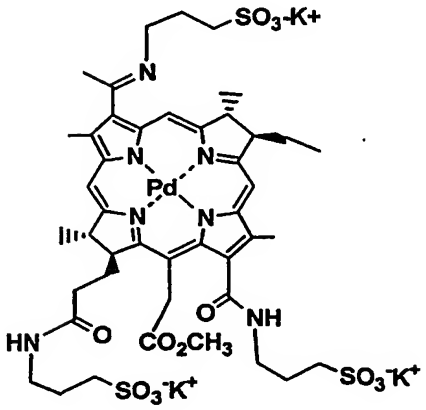
$H_2N\text{-}(CH_2)_3\text{-}SO_3H$
excess
DMSO/K-phosphate buffer



9

Scheme 1

CLAIMS:

1. A bacteriochlorophyll derivative containing at least two negatively charged groups and/or acidic groups that are converted to negatively charged groups at the physiological pH, excluding the compounds devoid a central metal atom and having a $-CH_2CH_2COOH$ group at position 17, $-CH_2COOH$ or –COOH group at position 15, a -COOH at position 13, a methyl group at positions 2, 7, 12, and 18, and an ethyl group at positions 3 and 8.

2. A bacteriochlorophyll derivative according to claim 1 containing two negatively charged groups.

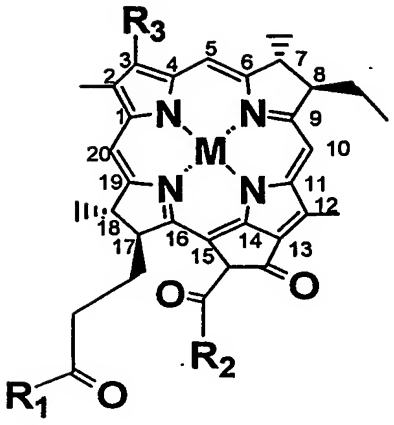
3. A bacteriochlorophyll derivative according to claim 1 containing three negatively charged groups.

4. A bacteriochlorophyll derivative according to any one of claims 1 to 3 wherein said negatively charged groups are selected from COO^-, COS^-, SO_3^-, and/or PO_3^{2-}.

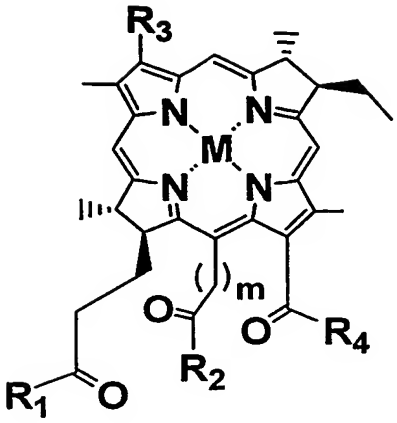
5. A bacteriochlorophyll derivative according to any one of claims 1 to 5 wherein said acidic groups that are converted to negatively charged groups at the physiological pH are selected from COOH, COSH, SO_3H, and/or PO_3H_2.

6. A bacteriochlorophyll derivative according to any one of claims 1 to 5 derived from a natural or synthetic derivative of bacteriochlorophyll, including compounds in which the central Mg atom has been deleted or replaced by other metal atoms.

7. A bacteriochlorophyll derivative according to claim 1 of the formula I or II:



(I)



(II)

wherein

M represents 2H or a metal atom selected from divalent Pd, Pt, Co, Sn, Ni, Cu, Zn and Mn, and trivalent Fe, Mn and Cr;

R_1, R_2, and R_4 each is Y- R_5;

Y is O, S or NR_5R_6;

R_3 is selected from $-CH=CH_2$, $-C(=O)-CH_3$, -C(=O)-H, $-CH=NR_7$, $-C(CH_3)=NR_7$, $-CH_2-OR_7$, $-CH_2-SR_7$, $-CH_2-NR_7R'_7$, $-CH(CH_3)-OR_7$, $-CH(CH_3)-SR_7$, $-CH(CH_3)-NR_7R'_7$, $-CH(CH_3)Hal$, $-CH_2-Hal$, $-CH_2-R_7$, $-CH=CR_7R'_7$, $-C(CH_3)=CR_7R'_7$, $-CH=CR_7Hal$, $-C(CH_3)=CR_7Hal$, and $-C\equiv CR_7$;

R_5 , R_6 , R_7 and R'_7 each is independently H; a straight or branched, saturated or unsaturated, substituted or unsubstituted hydrocarbon chain having from 1 to 25 carbon atoms and optionally interrupted by heteroatoms and/or carbocyclic or heterocyclic moieties; a residue of an amino acid, a peptide or of a protein; and, when Y is O or S, R_5 may further be H or R_8^+;

m is 0 or 1;

R_8^+ is H^+ or a cation;

provided that: (i) at least two of R_5, R_6, R_7 and R'_7 are such a hydrocarbon chain substituted by a negatively charged group or by an acidic group that is converted to a negatively charged group at the physiological pH and/or a residue of an amino acid, a peptide or of a protein; or (ii) at least two of R_1, R_2, and R_4 are OH, SH, $O^- R_8^+$ or $S^- R_8^+$; or (iii) at least one of R_1, R_2, and R_4 is OH, SH, $O^- R_8^+$ or $S^- R_8^+$ and at least one of R_5, R_6, R_7 and R'_7 is such a hydrocarbon chain substituted by a

negatively charged group or by an acidic group that is converted to a negatively charged group at the physiological pH and/or a residue of an amino acid, a peptide or of a protein, but excluding the compounds of formula II wherein M is 2H, R_3 is -C(=O)CH_3, R_1, R_2 and R_4 are OH, and m is 0 or 1.

8. A bacteriochlorophyll derivative according to claim 7 wherein said negatively charged groups are selected from COO^-, COS^-, SO_3^-, and/or PO_3^{2-}.

9. A bacteriochlorophyll derivative according to claim 7 wherein said acidic groups that are converted to negatively charged groups at the physiological pH are selected from COOH, COSH, SO_3H, and/or PO_3H_2.

10. A bacteriochlorophyll derivative according to claim 9 wherein R_1 is Y- R_5 ; Y is O, S or NH; and R_5 is a hydrocarbon chain substituted by functional groups selected from OH, SH, SO_3H, NH_2, $CONH_2$, COOH, COSH, PO_3H_2.

11. A bacteriochlorophyll derivative according to claim 7 wherein R_5 is the residue of an amino acid, a peptide or a protein.

12. A bacteriochlorophyll derivative according to any one of claims 1 to 11 wherein M is Pd.

13. A bacteriochlorophyll derivative of formula II in claim 6 wherein:

M represents 2H, divalent Pd, Cu, or Zn, or trivalent Mn;

R_1 is $-O^- R_8^+$, $-NH-(CH_2)_n-SO_3^- R_8^+$, or Y-$R_5$ wherein Y is O, S or NH and R_5 is the residue of an amino acid, a peptide or a protein;

R_2 is (C1-C6) alkoxy;

R_3 is $-C(=O)-CH_3$, $-CH=N-(CH_2)_n-SO_3^- R_8^+$; or $-CH_2-NH-(CH_2)_n-SO_3^- R_8^+$;

R_4 is $-NH-(CH_2)_n-SO_3^- R_8^+$; $NH-(CH_2)_n-COO^- R_8^+$; $NH-(CH_2)_n-PO_3^{2-} (R_8^+)_2$;

R_8^+ is a monovalent cation;

m is 1, and n is an integer from 1 to 10.

14. A bacteriochlorophyll derivative according to claim 13, consisting of the compound palladium 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(2-sulfo-ethyl) amide dipotassium salt.

15. A bacteriochlorophyll derivative according to claim 13, consisting of the compound 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(2-sulfoethyl)amide dipotassium salt

16. A bacteriochlorophyll derivative according to claim 13, consisting of the compound palladium 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1,17^3-di(3-sulfo-propyl)amide dipotassium salt

17. A bacteriochlorophyll derivative according to claim 13, consisting of the compound palladium 3^1-(3-sulfopropylimino)-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide tripotassium salt.

18. A bacteriochlorophyll derivative according to claim 13, consisting of the compound copper(II) 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(2-sulfoethyl) amide dipotassium salt.

19. A bacteriochlorophyll derivative according to claim 13, consisting of the compound zinc 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(2-sulfoethyl) amide dipotassium salt.

20. A bacteriochlorophyll derivative according to claim 13, consisting of the compound manganese(III) 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(2-sulfo-ethyl)amide dipotassium salt.

21. A bacteriochlorophyll derivative according to claim 13, consisting of the compound palladium 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(2-sulfoethyl) amide, 17^3-(N-immunoglobulin G) amide potassium salt.

22. A bacteriochlorophyll derivative according to claim 13, consisting of the compound palladium 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(2-carboxy-ethyl)amide dipotassium salt.

23. A bacteriochlorophyll derivative according to claim 13, consisting of the compound palladium 3^1-oxo-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1-(3-phospho-propyl)amide tripotassium salt.

24. A bacteriochlorophyll derivative according to claim 13, consisting of the compound palladium 3^1-(3-sulfopropylamino)-15-methoxycarbonylmethyl-rhodobacteriochlorin 13^1,17^3-di(3-sulfopropyl)amide tripotassium salt.

25. Intermediates for the preparation of the compounds of the invention consisting of:
Palladium bacteriopheophorbide a 17^3–(3-sulfo-1-oxysuccinimide) ester sodium salt, and
Palladium bacteriopheophorbide a 17^3-(3-sulfopropyl)amide potassium salt.

26. A pharmaceutical composition comprising a bacteriochlorophyll derivative according to any one of claims 1 to 24, and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition according to claim 26, wherein said bacteriochlorophyll derivative is of the formula I or II in claim 7.

28. A pharmaceutical composition according to claim 27, comprising a bacteriochlorophyll derivative as defined in any one of claims 8 to 24.

29. The pharmaceutical composition according to any one of claims 26 to 28 for photodynamic therapy.

30. The pharmaceutical composition according to claim 29 for vascular-targeting photodynamic therapy.

31. The pharmaceutical composition according to any one of claims 26 to 30 for photodynamic therapy of tumors.

32. The pharmaceutical composition according to claim 31 for photodynamic therapy of melanoma, colon or prostate cancer.

33. The pharmaceutical composition according to any one of claims 26 to 30 for photodynamic therapy of age-related macular degeneration.

34. The pharmaceutical composition according to any one of claims 26 to 30 for photodynamic therapy of benign prostate hypertrophy.

35. A pharmaceutical composition according to any one of claims 26 to 30 for killing cells or infectious agents comprising bacteria and viruses.

36. A pharmaceutical composition according to claim 26 for in vitro killing of cells or infectious agents comprising bacteria and viruses in a biological product upon illumination of said product.

37. The pharmaceutical composition according to claim 36 wherein said biological product is blood.

38. In a method for photodynamic therapy using a photosensitizer, the improvement wherein said photosensitizer is a bacteriochlorophyll derivative according to any one of claims 1 to 24.

39. In a method for diagnosis of tumors using a photosensitizer, the improvement wherein said photosensitizer is a bacteriochlorophyll derivative according to any one of claims 1 to 24.

40. In a method for killing of cells or infectious agents comprising bacteria and viruses, using a photosensitizer, the improvement wherein said photosensitizer is a bacteriochlorophyll derivative according to any one of claims 1 to 24.

41. A method according to claim 40, for killing of infectious agents in biological products.

42. A method according to claim 41, wherein said biological product is blood.

For the Applicants,

Paulina Ben-Ami

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BEN-AMI & ASSOCIATES
Patent Attorneys

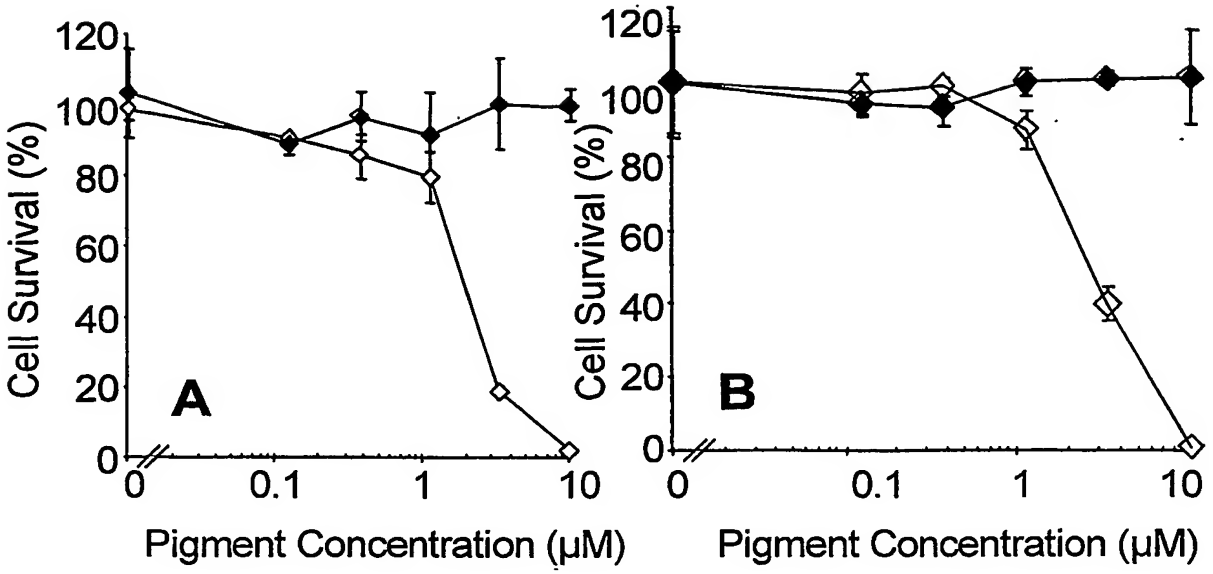

Figure 1

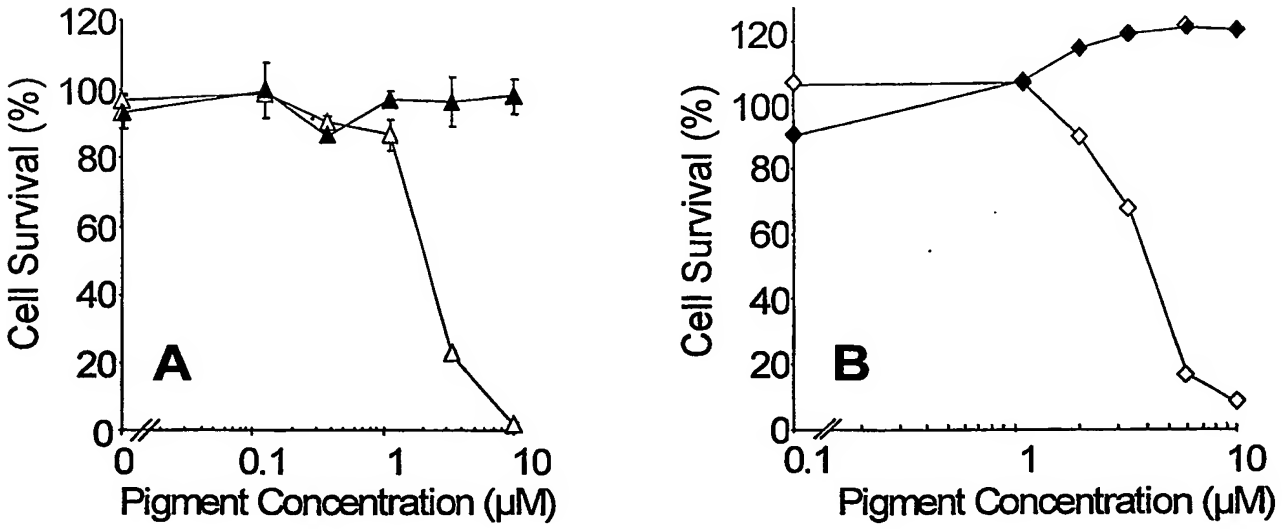

Figure 2

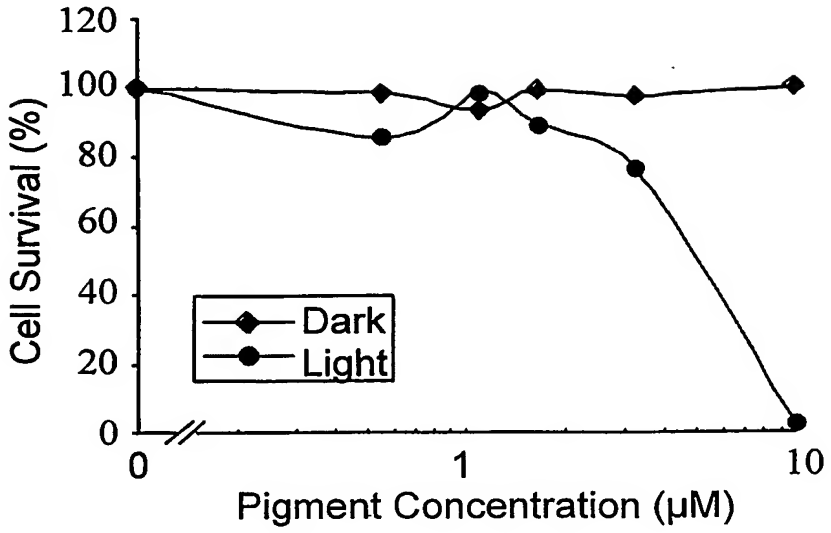

Figure 3

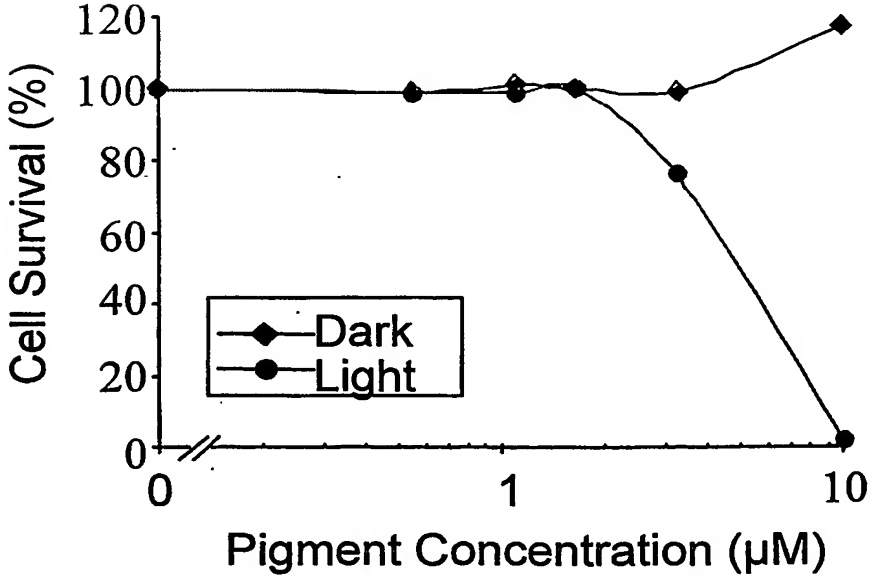

Figure 4

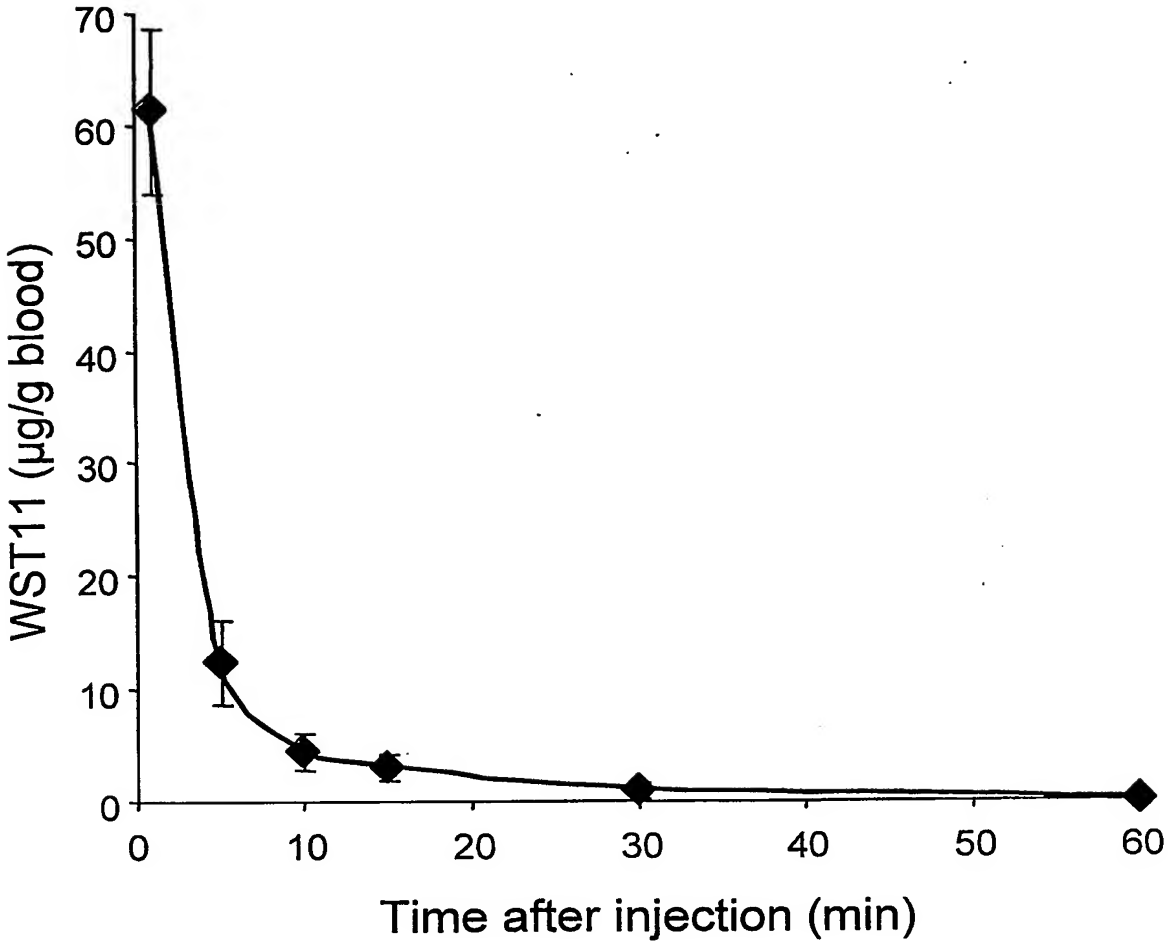
70
60
50
40
30
20
10
0
WST11 (µg/g blood)
0
10
20
30
40
50
60
Time after injection (min)

Figure 5

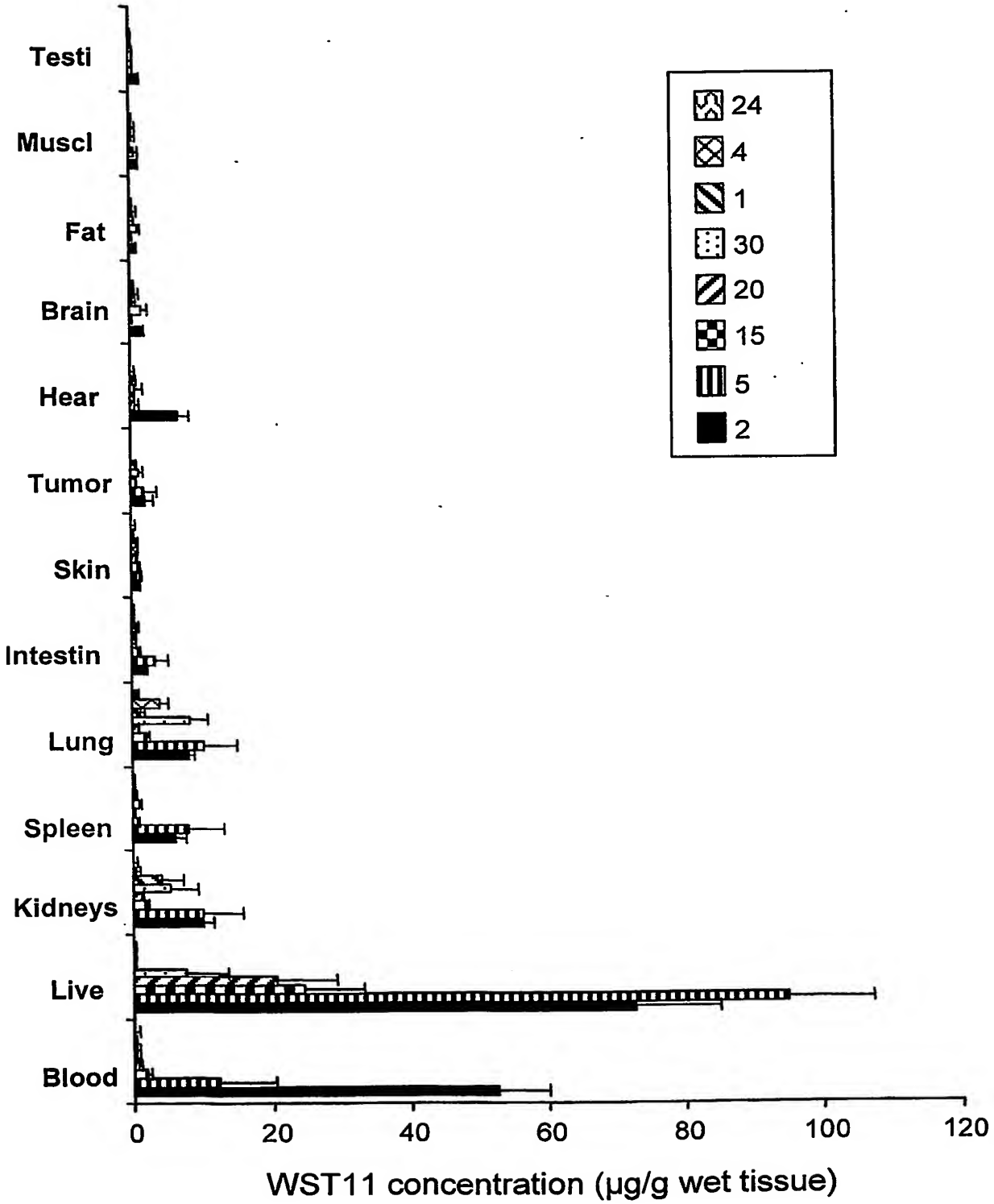
Testi
Muscl
Fat
Brain
Hear
Tumor
Skin
Intestin
Lung
Spleen
Kidneys
Live
Blood
24
4
1
30
20
15
5
2
0
20
40
60
80
100
120
WST11 concentration (µg/g wet tissue)

Figure 6

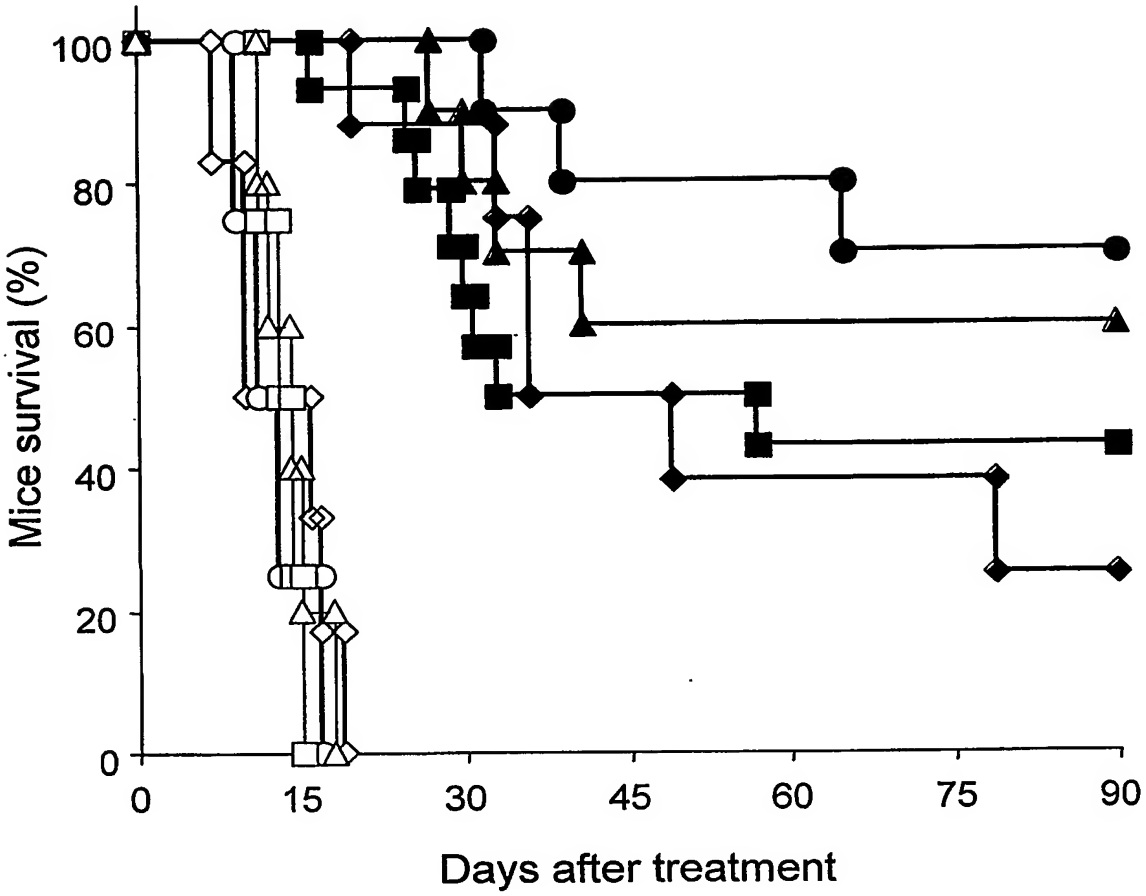
100
80
60
40
20
0
Mice survival (%)
0
15
30
45
60
75
90
Days after treatment

Figure 7

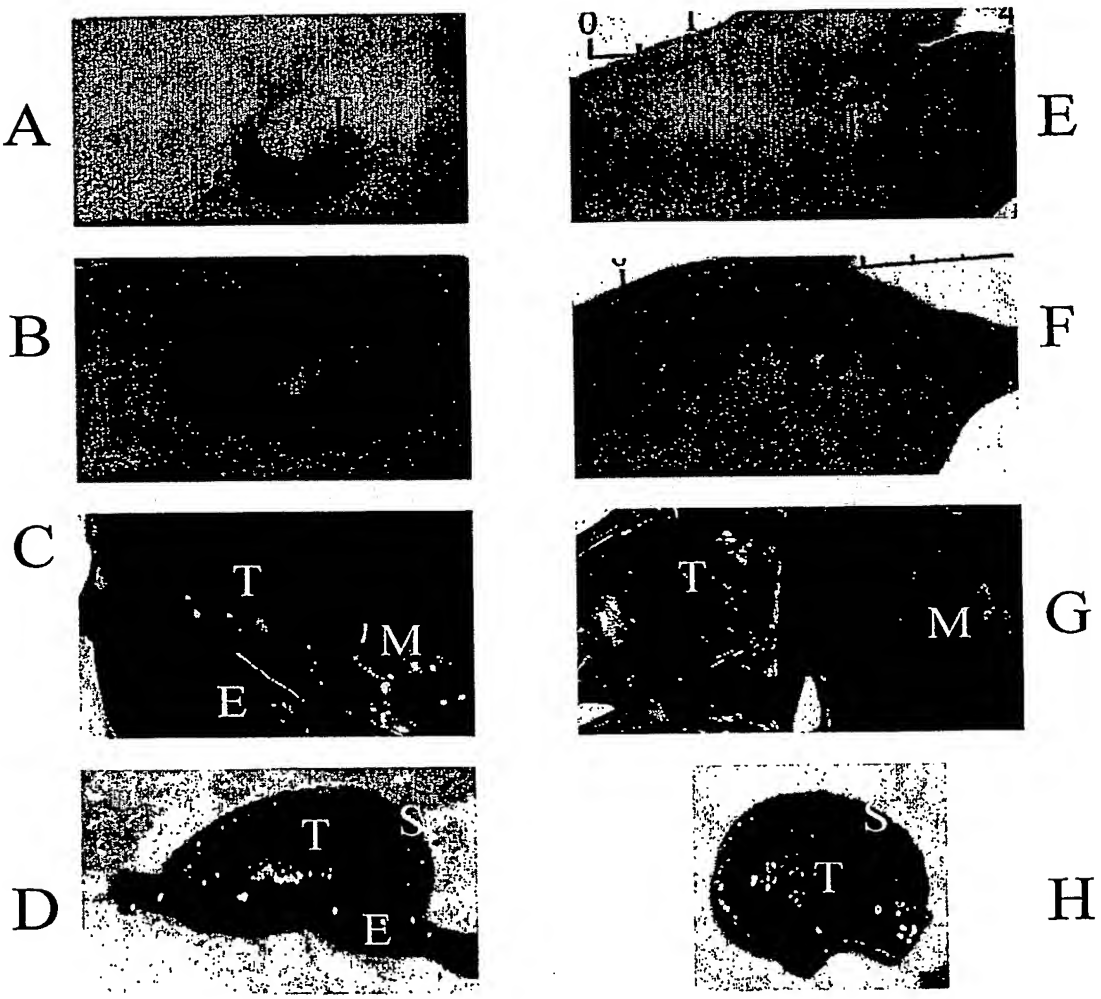

Figure 8

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